in exercised animals was observed in this laboratory in experimental *Trypanosoma cruzi* myocarditis of mice(11).

Summary. A cardiotropic strain of Coxsackie A9 virus was inoculated intraperitoneally into groups of adult C_3H mice, one-half of which were vigorously exercised daily by swimming. Uninfected controls were studied in parallel. Among the infected mice, the virus was isolated in high titer from the hearts of a significantly greater proportion of those that were exercised. The possible mechanisms by which exercise could augment replication of virus in the myocardial tissue were discussed. On the ninth day after inoculation, the weight and relative weight of the heart were significantly greater in the exercised mice than in those that were not exercised. Among the exercised animals, the the weight and relative weight of the heart were significantly greater in those that were infected with virus than in those that were not infected. The increase in weight could not be attributed to increased water content or to inflammatory infiltrate.

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Failure of Actinomycin D to Inhibit Antitoxin Production to a Challenging Injection of Antigen.*† (29697)

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Protein synthesis requires the presence of a specific mRNA, coded directly from DNA, which in bacteria appears to have a rapid rate of turnover(1). This is in contrast with studies in higher forms which suggest the existence of a long lived mRNA(2-6). Antibody is also synthesized by an mRNA coded from DNA(7,8,9) but it appears to require a specific antigen to stimulate its production. In addition, the antigen establishes an "immunological memory" which leads to an augmentation of the gamma globulin production upon re-exposure. This augmentation must be due either to a rapid assembling of protein synthesizing units or to an accumulation of such units in the cells. Since nucleic acids may be involved, it is possible to determine their role by the use of nucleic acid inhibitors. Actinomycin D has been shown to inhibit DNA-dependent RNA synthesis(10). It was therefore of interest to determine if such an inhibition would affect antibody production in response to an injection of specific antigen in immunized mice.

Materials and methods. The animals used were adult BDF^1/Jax mice, weighing approximately 25 g. They were immunized by 7 weekly subcutaneous injections of tetanus toxoid. The last sensitizing injection was given 9 months prior to the onset of drug treatment in order to obtain animals with a

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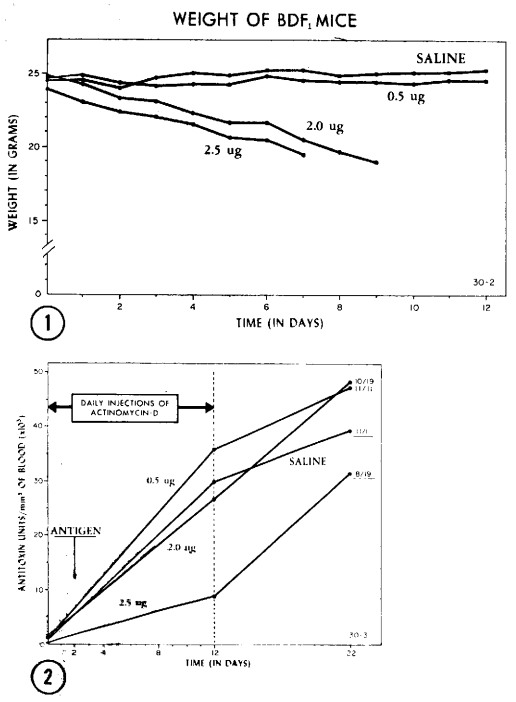

FIG. 1. Effect of Actinomycin D on body weight of BDF_1 mice. Weight records of groups receiving 2.0 μg and 2.5 μg were maintained only to the date of the first death in each group.

FIG. 2. Antitoxin titers in immunized mice treated with Actinomycin D. Numbers on the right indicate the proportion of animals surviving the Actinomycin D treatment.

high degree of sensitization and low antitoxin titers.

The toxicity of Actinomycin D was determined in these mice using weight loss as well as lethality as criteria. Twenty-four mice were divided into 4 equal groups and injected daily, s.c. with 0.2 ml of solution containing either 0.5 μg, 2.0 μg or 2.5 μg Actinomycin D dissolved in nonpyrogenic saline. Each animal was weighed daily throughout the drug treatment.

The remaining 60 animals were divided into 4 groups and injected with the same solutions as above. Immediately after the 3rd injection of Actinomycin D, these animals were given an i.p. injection of 0.2 ml of tetanus toxoid (Aluminum-Phosphate-Adsorbed, Lederle). Blood samples were taken from the tail vein prior to the drug treatment (Day 0) and 10 days after the challenging injection of antigen (Day 12). The drug injections were then terminated and the animals allowed to recover for 10 days at which time another sample of blood was taken (Day 22). Antitoxin levels were determined by bioassay against 10 mld tetanus toxin(11).

Results. The results of the toxicity test are shown in Fig. 1. It can be seen that injections of 0.5 μg of Actinomycin D had no appreciable effect on total body weight as compared with the saline controls. Injections of 2.0 μg, on the other hand, resulted in an average weight loss of 6 g during the first 10 days. Injections of 2.5 μg showed similar weight losses and the first death occurred after eight days. Approximately 50% of the animals receiving 2.0 μg and 2.5 μg Actinomycin D daily were dead by the 12th day.

The antitoxin titers in the BDF_1 mice which had been given a challenging injection of antigen during the Actinomycin treatment can be seen in Fig. 2. All animals in the groups receiving 0.5 μg and in the saline controls survived, while 10 out of 19, and 8 out of 19 survived in the groups receiving 2.0 μg and 2.5 μg respectively. Both the control and experimental groups responded to the reinjection with significant increases in antitoxin titers. The animals receiving 0.5 μg Actinomycin D had higher titers with somewhat greater variations than the saline injected controls. Animals injected with 2.0 μg had slightly lower titers, and those injected with 2.5 μg had titers which were markedly lower than the saline controls. Actinomycin, in all dose levels administered, resulted in toxic manifestation in the area of injection, including loss of hair and marked signs of necrosis. After 13 consecutive daily injections the surviving animals were allowed to recover and the antitoxin titers determined on day 22. In both control and experimental groups the antitoxin titers had continued to increase, with the highest rate of increase found in animals injected with 2.0 μg and 2.5 μg of the Actinomycin.

Discussion. The results obtained indicate that Actinomycin D, in doses sufficiently large to cause marked weight loss and death, did not prevent previously sensitized recipient mice from responding to a reinjection of antigen with the production of specific anti-

body. Actinomycin D in doses of 2.0 μg and 2.5 μg were definitely toxic to the recipients as evidenced by the characteristic symptoms of alopecia, stomatitis, splenic emaciation, etc. Although animals receiving these doses were not able to maintain their body weight, they were able to produce large quantities of antitoxin following a challenging injection of antigen. These data are in agreement with Wust *et al*(12) who reported that after single injections of Actinomycin D a delay occurred in the appearance of antibody but there was no reduction in the total antibody produced. The data obtained with .5 μg Actinomycin are similar to those of Smiley *et al*(8) who reported that low doses of Actinomycin D may produce slightly higher antibody titers than control animals. Since it has been pointed out that extremely low concentrations of the drug are sufficient for an inhibitory effect on DNA-dependent RNA synthesis(13,14), it is highly likely that doses of Actinomycin D sufficiently high to cause weight loss and death would also inhibit synthesis of mRNA.

At least 2 possible explanations exist for the appearance of antibody after a challenging injection in Actinomycin treated mice: A) the antibody present was stored in the animals in a bound state and released thereafter; or B) the templates for the production of specific antibody exist in an inactive form in immunized animals, and are merely reactivated by the presence of antigen. The first possibility is not likely since, as shown by Taliaferro(15) and Stavitsky(16), labeled amino acids are rapidly incorporated into antibody after a challenging injection, indicating de novo synthesis.

In view of the amount of antibody produced and the continued production over several weeks, it seems likely that antibody production must be due to an activation of a mechanism for synthesis of this particular protein. Gamma globulin synthesis, like that of all protein synthesis is dependent upon the presence of a specific mRNA associated with ribosomes in the presence of activated amino acids in the form of aminoacyl-sRNA(8,9,10). The mRNA in turn is synthesized in accordance with a specific code present in the genome of the cell. Since new mRNA production is inhibited by Actinomycin D, it appears likely that the specific mRNA needed for antibody production must be present in an inactivated form prior to the antigen reinjection. Speirs(17) has suggested that one molecule of antigen can inactivate a specific mRNA forming an antigen-RNA complex. This complex can be passed from cell to cell and results in a sensitization of the cell to specific antigen. Reexposure to the antigen results in a breaking up of the complex and a release of mRNA which is utilized for antibody production.

Experiments concerning long-lived mRNA are not all based upon the same criteria for longevity. Bell and coworkers(4,5,6) have reported RNA fractions which can function for long periods before degradation. On the other hand, Gross and Cousineau(18) have reported a protein-synthesizing mechanism in sea urchin eggs which appears to lie dormant until activated by fertilization. This latter form, the inactivated mRNA, resembles the situation in immunity as described here. The mRNA appears to become activated by antigen in a manner similar to the activation of the sea urchin eggs by fertilization. This indicates that 2 types of persisting mRNA may be present: an activated mRNA with a long half life, and an inactivated mRNA which can exist for long periods prior to activation. It is possible that the inactivated mRNA may be responsible not only for immunological memory, but also for induced enzyme synthesis(19) and non-genomically controlled protein production(18).

Summary. Daily injections of Actinomycin D, which were sufficiently high to produce marked weight losses and other toxic manifestations, failed to eliminate antitoxin production to a challenging injection of tetanus toxoid. Since DNA-dependent RNA synthesis was presumably inhibited, the data support the existence of a persisting inactivated mRNA, in a form which is activated by the reinjection of antigen.

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Inhibition by Lipopolysaccharide (Endotoxin) of Antibody Response of Rabbit to Common Antigen of Enterobacteriaceae.* (29698)

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An antigen common to numerous, though not all, enteric, gram-negative bacilli was discovered by Kunin *et al*(1) by means of the hemagglutination test with *Escherichia coli* O14 antiserum. This common antigen (CA) readily attaches to erythrocytes, and the modified red blood cells are then specifically agglutinated in the presence of the corresponding antibody(1,2). Surprisingly, the antibody does not cause bacterial agglutination, although the common antigen is present on the surface of the cells, nor does it produce precipitation of soluble antigen, obtained from heated cultures. Antibodies against CA are readily engendered after intravenous injection of *E. coli* O14, but numerous other serogroups of *E. coli* and many species of enteric bacteria are not immunogenic(1). It was shown recently that injection of the antigen from bacteria other than *E. coli* O14, together with Freund's adjuvant, into the footpad of rabbits stimulates antibodies against CA in high titer(3). In contrast to the soluble antigen, CA attached *in vitro* to autologous or heterologous erythrocytes, L cells, or to lymphocytes from patients with leukemia, upon intravenous injection into rabbits, also elicits antibody formation(4,5). Suzuki *et al*(6) recently observed that CA from enteric bacteria other than *E. coli* O14 is soluble in 85% ethanol and that intravenous injection of this fraction readily engenders CA antibodies in the rabbit. Furthermore, injection of a mixture of the ethanol soluble and insoluble fractions results in a decreased or absent CA antibody formation(6). These observations suggested the

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